



Ring Trial on African Swine Fever Virus (ASFV) Real-Time PCR

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Total number of authors:
20

Publication date:
2011

Document Version
Publisher's PDF, also known as Version of record

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Citation (APA):

Fernandez-Pinero, J., Gallardo, C., Elizalde, M., Rasmussen, T. B., Stahl, K., Loeffen, W., Blome, S., Batten, C., Crooke, H., Le Potier, M-F., Uttenthal, Å., Leblanc, N., Michaud, V., Kowalczyk, A., Markowska-Daniel, I., Tignon, M., Iscaro, C., Giammarioli, M., Arias, M., & Hoffmann, B. (2011). *Ring Trial on African Swine Fever Virus (ASFV) Real-Time PCR*. Poster session presented at 5th Annual Meeting EPIZONE, Arnhem, Netherlands. <http://www.epizone-eu.net/annualmeeting/default.aspx>

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D 22: EPIZONE: RING TRIAL ON AFRICAN SWINE FEVER VIRUS (ASFV) REAL-TIME PCR

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Key words: African swine fever, real-time PCR, ring trial, diagnostics

As a part of the EPIZONE WP4.1: Real-time PCR diagnostics, a ring trial on real-time PCR for the detection of African Swine Fever Virus (ASFV) DNA was carried out.

The ring trial comprised a collection of 27 blind DNA samples to be tested by ASFV real-time PCR methods established at each participating laboratory, as well as by one common reference method. The aim of this study was the identification of useful ASFV real-time PCR assays, in terms of sensitivity, specificity, repeatability, and reproducibility.

The ring trial was organized by the European Union Reference Laboratory (URL) for African Swine Fever (ASF), CISA-INIA, Valdeolmos, Spain, with assistance from the other ASF core group members within the EPIZONE WP4.1: DTU, Lindholm, Denmark, and SVA, Uppsala, Sweden. A total of 12 laboratories took part in the ring trial, representing nine ASF National Reference Laboratories of EU member countries and some other EPIZONE partners working on ASF.

Clinical material collected from experimentally ASFV infected pigs or ASF viral suspensions were diluted in healthy donor pig material in order to prepare the sample panel. The coded DNA collection finally comprised 23 porcine serum, blood and tissue samples containing different amounts of reference ASFV isolates representative of genotypes I, II, IX, and X, and 4 ASFV negative samples. A primer/probe mix containing both primer set and TaqMan probe described by King et al (2003), and the URL SOP based on the aforementioned method (considered as the reference assay in this study) were provided also to the participants.

All 12 participants performed the real-time PCR recommended in the OIE Manual (described by King et al, 2003), either following the provided URL SOP (8x), other modified in-house procedures (2x) or both (2x). One lab ran a modified procedure of the PCR method described by Zsak et al, 2005. Two labs tested the only currently commercially available ASFV PCR kit (Tetracore Inc., Rockville, MD, US), which includes all reagents dried down, as well as a rehydration buffer and a positive control. Seven additional in-house-developed real-time PCR methods were carried out by eight participating labs, though all unpublished so far. It is noteworthy that these new techniques are based on different molecular approaches: FRET probes, ASF/CSF triplex PCR, conventional TaqMan probe, PriProET system, LATE-PCR technology, SybrGreen, and UPL commercial probes. In addition, it is remarkable that all the PCR methods reported in the ring trial are based on the amplification of a DNA fragment within the VP72 ASFV genome region.

All PCR systems worked rather well and were capable of detecting the range of viral p72 genotypes considered in the study. However, the methods recently developed generally increased the sensitivity and repeatability of the King-based PCR assays. Furthermore, the commercial ASFV PCR kit also demonstrated a fine sensitivity in the different platforms employed.

In conclusion, a range of valuable molecular tools are currently in use for the ASFV detection, including one commercial kit, although most of them are not yet published.

Acknowledgements: This work has been funded by the EU Network of Excellence EPIZONE (contract no. FOOD-CT-2006-016236) and by the ASF EU Reference Laboratory funds (DG-SANCO-EC). The excellent technical work carried out by A. Robles, C. Gómez, and A. Villalba is gratefully recognized.

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